THE REDUCING DIVISIONS IN THE SPERMATOGENESIS OF DESMOGNATHUS FUSCA.

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DESPITE the fact — perhaps rather because of it — that the spermatogenesis of salamanders was the first to receive a careful investigation, and has since been twice made the subject of rather monographic treatment, there exist at present considerable confusion and disagreement in the results of the different investigators.

Flemming,[1] in 1887, in his paper in which he recognized two divergent types of mitosis to which he attached considerable significance, the "heterotypic" and "homotypic," gave the following scheme of spermatogenesis in *Salamandra:* (1) a period of multiplication of the cells; (2) a period of growth in which are formed the spermatocytes (of the first generation), large cells; these, by a division proceeding generally, though not universally, according to the heterotypic plan, form daughter-cells of medium size; and these, by another division, generally homotypic, sometimes heterotypic, form small (granddaughter) cells which are directly transformed into the spermatozoa.

Vom Rath,[2] in 1893, followed with a paper upon the spermatogenesis of *Salamandra*, in which he added to the three generations of cells recognized by Flemming three others, in the last two of which occurred a reduction by means of tetrad formation, which, after the appearance of Flemming's work on *Salamandra*, had been already observed in several invertebrates, and by vom Rath[3] himself in the mole-cricket (*Gryllotalpa vul-*

[1] Flemming, W., "Neue Beiträge zur Kenntniss der Zelle. I. Die Kerntheilung bei den Spermatocyten von Salamandra maculosa," *Arch. f. mikr. Anat.* Vol. xxix, p. 389. 1887.

[2] Vom Rath, O., "Beiträge zur Kenntniss der Spermatogenese von Salamandra maculosa," *Zeitschr. f. wiss. Zool.* Vol. lvii. 1893.

[3] Vom Rath, O., "Zur Kenntniss der Spermatogenese von Gryllotalpa vulgaris," *Arch. f. mikr. Anat.* Vol. xl, pp. 102–132. 1892.

garis); so that the way was well paved for the recognition of similar results in *Salamandra* as well.

Meves,[1] finally, after a careful reinvestigation of the spermatogenesis of *Salamandra*, has found that Flemming was right as to the number of cell generations, and vom Rath wrong; that there is no tetrad formation and no reduction in the Weismannian sense; both of the final divisions being equation divisions; the first heterotypic in character, the second homotypic — not mixed, as Flemming had thought.

The large number of forms in which tetrad formation or its equivalent has since been found to occur might suggest that vom Rath is nevertheless right, or partially right, and Meves wrong. My results, however, upon the American salamander *Desmognathus fusca* confirm Meves's interpretations in all the essential points; no tetrad formation occurs, unless exceptionally, and the two final divisions recognized by Flemming are both equation divisions. Though this is the case, there yet exists the possibility of a qualitative reduction, as will be indicated below.

After the last division of the spermatogonia there occurs a well-marked synapsis stage, such as has been recognized by Moore[2] in Elasmobranchs, in which the detailed structure of the nucleus becomes very difficult to make out. The chromatin emerges from the synapsis in the form of a fine, intricately coiled thread or threads (it has not yet been ascertained which), and the nucleus enters on its period of growth preparatory to the first of the two "reducing" divisions. During this period the chromatin thread (or threads) shortens and thickens, and finally may be resolved into twelve horseshoe-shaped loops, which, in most cases at least, are so arranged as to have their ends — the open side of the loop — opposite the centrosphere, with its centrosome, now precociously divided (Fig. 1).

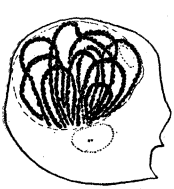


FIG. 1.

The division into which the nucleus is about to enter pro-

[1] Meves, Fr., "Ueber die Entwicklung der männlichen Geschlechtszellen bei Salamandra maculosa," *Arch. f. mikr. Anat.* Vol. xlviii, pp. 1–83. 1896.

[2] *Quart. Journ. Micr. Sci.* Vol. xxxviii, pp. 275. 1895–96.

ceeds according to the heterotypic plan, as both Flemming and Meves have already described in *Salamandra*. The twelve segments lose their position relative to the centrosphere, split longitudinally and incompletely (or, according to Meves, completely but with a subsequent fusion of their ends; I have not been able to determine this satisfactorily), and by a shortening and thickening become converted into rings, loops, or by twisting into 8's, as has been so often described and figured since the appearance of Flemming's paper in 1887. The split chromatin segments, in the form of rings or loops, generally more or less irregular, take up a peripheral position in the nucleus under the nuclear membrane. The centrosomes diverge within the centrosphere, the spindle is formed between them, in which the chromatin rings take up their position, forming the figure so characteristic of heterotypic mitosis. In the anaphase, as the daughter-chromosomes are passing to the poles, a second precocious, longitudinal splitting takes place, as recognized by Flemming. As the chromosomes approach the poles, however, they become so closely massed (possibly fused ?) that it has been impossible to trace the continued existence of this splitting. In the telophase they become again separated from each other, still retaining their arrangement in relation to the pole (centrosome) (Fig. 2), the apices of the *V*'s all turned in the same direction.

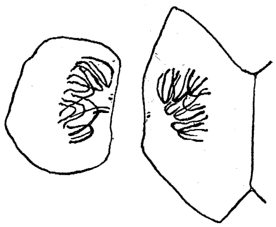


FIG. 2.

The nucleus of the spermatocyte of the second order does not go into a true resting stage, but the chromosomes remain distinct and easily distinguishable. Each loop is furthermore longitudinally split, so that there are twenty-four (presumably), which, however, are not entirely separate and independent, but are joined together in twos at the apices of the *V*'s (Fig. 3).

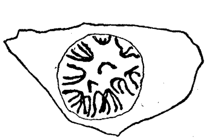


FIG. 3.

I believe these pairs are undoubtedly the incompletely split chromosomes of the anaphase in the previous division, although their close massing in the late anaphase has rendered it so far

impossible to trace them. The longitudinal splitting may have been complete, and the fusion of the apices been of secondary occurrence. The two chromatin segments so joined in pairs at their middle points show a tendency to diverge from each other, and in most cases, therefore, each pair is formed of four chromatin threads radiating from the point of fusion and lying in two planes at right angles to each other, as indicated in Fig. 4. There follow a shortening and thickening of the chromatin threads by which each pair becomes converted into a + or an X.

These usually tend to lie near the periphery of the nucleus under the nuclear membrane, as do the rings in the corresponding stage in the spermatocyte of the first order. Finally, the fusion is dissolved and there are formed from each + or X two *V*'s, daughter-chromosomes of the second division. These are generally quite irregularly distributed at first; soon, however, they take up their position in the center of the cell, to form a somewhat loose equatorial plate in which the daughter-chromosomes are generally quite widely separated from each other. Migration to the two poles to form the nuclei of the spermatids occurs in *Desmognathus* in the manner already described by Flemming and Meves for *Salamanda.*

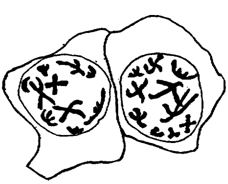


Fig. 4.

The early splitting and early and complete separation of the chromosomes in the spermatocyte of the second order was recognized by Flemming and made a characteristic of the peculiar form of mitosis which he called "homotypic," basing the type on the conditions found in the second division in *Salamandra.*

Although it seems to me probable that the X's and their dissolution into *V*-shaped chromosomes is due simply to an incomplete precocious longitudinal splitting of the chromosomes, which upon its final completion gives an equation division of the chromatin mass represented by the formula $\frac{a-b}{a-b}$, thus, $\frac{a\ b}{a\ b}$, it is nevertheless possible that the final separation is represented by $\frac{a-a}{b-b}$, thus, $\frac{a\ b}{a\ b}$; and therefore it

would be a qualitative, reducing division in the sense of Weismann. No absolute decision between these two possibilities could be arrived at; nor does it seem to me likely that a determination of the way in which the separation actually occurs may be gained.

I know of no results on other forms that furnish circumstantial evidence in favor of a qualitative reduction taking place in the manner suggested above as possible. Meves, it should be remembered, found that in *Salamandra* the second division was a qualitative equation division, and did not describe anything corresponding to the X formation that occurs in *Desmognathus*.

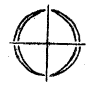
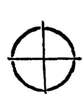
The union of chromosomes, or daughter-chromosomes, in pairs, whether with a reduction of the number one-half or not, is conceded by most as furnishing a basis for a qualitative reduction. Typically (perhaps) the reduction is accomplished by the union in pairs of the chromosomes before the first splitting; there being a reduction of the number to one-half and a second longitudinal splitting being wanting; or (Calkins[1] in *Lumbricus*) conjugation may take place after the first longitudinal splitting, and reduction follow, as in the typical case. According to Lee,[2] in *Helix* a longitudinal splitting of the chromosomes, separation of the daughter-chromosomes, and a subsequent fusion (so far resembling Korschelt's[3] results on *Ophryotrocha*) take place before the divisions of the spermatocyte, of which the first is longitudinal, the second transverse; there is thus no reduction in the number of the chromosomes, but there is a quantitative and qualitative reduction in the second division, the latter depending on the heterogeneous conjugation of chromosomes before the first division.

If the second division in *Desmognathus* is to be looked upon as a "reducing" division, it may be considered in two ways. The original union of the chromosomes, after two longitudinal splittings of the united chromosomes, is dissolved and a new union between the daughter-chromosomes established; or,

[1] *Journ. Morph.* Vol. xi, pp. 271–302. 1895.

[2] *La Cellule.* Vol. xiii, pp. 201–270. 1897.

[3] *Zeitschr. f. wiss Zool.* Vol. lx, pp. 543–688. 1895.

from the standpoint of the more typical mode of reduction by tetrad formation with longitudinal and transverse divisions, there would occur in *Desmognathus* a reduction in number to one-half, a longitudinal (equation) division, followed by an attempt at a second longitudinal division, which, however, is not completed, and is prevented from being completed, by the second division, which is transverse.  Shorten the interval elapsing between the first and second divisions, and (possibly thereby) eliminate the second longitudinal splitting, and the process is reduced to the typical form.  It seems to the writer, however, far more likely that both divisions in *Desmognathus* are equation divisions, in agreement with the results of Brauer, Hertwig, Moore, Meves, and the majority of the botanical workers.

There are two or three interesting comparisons that may be made between the first and second divisions of the spermatocyte. In the period of growth to form the spermatocyte of the first order the chromatin segments form loops or *U*'s with the open end toward the centrosphere and centrosome (Fig. 1). In the spermatocyte of the second order, on the other hand, the apices of the *V*'s are toward the centrosome. In the longitudinal division of the segments in the spermatocyte of the first order the free ends of the segments remain united (or fuse after separating), forming rings.  In the spermatocyte II the apices (opposite ends of the joined chromosomes) remain united, and the corresponding figure in the second division is a cross.  The chromatin in the two divisions is thus contrasted in these two particulars, to which it is felt some importance may attach.

In *Desmognathus*, therefore, there are two divisions intervening between the last spermatogone division (as so determined) and the spermatid, in both of which occurs longitudinal split-

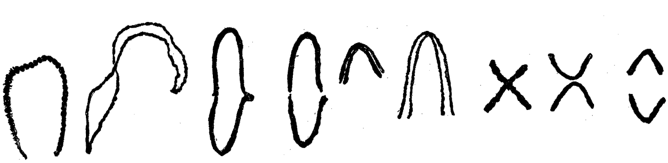


FIG. 5

ting of the chromatin segments, there being thus agreement with the results of Flemming and Meves on the European form *Salamandra*, as opposed to those of vom Rath. The possibility of a reducing division is nevertheless believed to exist. The diagram on the preceding page sets forth the author's interpretation of the division of the chromosomes in the last two divisions.

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